Neisseria species (such as *Neisseria meningitidis*), Enterococcus species (such as *Enterococcus fecalis*, *Enterobacter cloacae*) *Escherichia coli* and *Klebsiella pneumoniae*.

Example 7 - Typing of bacteria by dideoxy DNA-sequencing

The general approach of example 6 was repeated, and dideoxy (Sanger) DNA-sequencing was used as the sequencing method. The primer pool comprising 19b/31b (19b binds to *Listeria monocyogenes*, 31b binds to *Haemphilus influenzae*. The results show that Sanger sequencing is also suitable to use for analysis of typing with the set of primers of the invention. Fig 12 shows sequencing *Listeria monocyogenes* by sequencing primers of invention. An ABI prism DNA analyzer 3700 (Applied biosystems) was used for the DNA-sequencing with Big dye terminator kit according the manufacturer's manual. This work is in press in journal of Molecular and Cellular Probes.

Claims

My claims for the invention are:

1 The multiple sequencing oligonucleotide primer pool method is utilized for specific genotyping/typing/identification/detection/sequencing of a sample of nucleic acid molecules, whereby i) the molecules are suspected to contain at least one type/species/target of a target/variable region or region of interest, each type having different nucleotide patterns ii) the molecules are suspected to contain unspecific amplification in the amplification product and comprising the steps of:

(a) Providing the sample is of nucleic acid molecules;

(b) Providing a mixed set of at least two sequencing oligonucleotide primers, whereby each primer is designed for being specific for one type/species/group/target chosen from the known set of types/target of the nucleic acid sample, thereby allowing a primer, which is specific for a type/species/group/target that is present in the sample, to hybridize in or close to the target/variable region or region of interest;

(c) Mixing the set of sample and primers under conditions allowing a primer or primers to hybridize if a type or types that is specific for the primer or primers is present in the sample;

(d) Determining the type/species/target region to which the primer or primers has hybridized by extending the hybridized primer(s) in a DNA-sequencing reaction.

2 Method according to claim 1, wherein the sequencing reaction is performed by sequencing-by-synthesis, dideoxy sequencing (sanger), sequencing by mass spectrometry or any other DNA sequencing technology, applicable to the method of invention.

3 Method according to any one of claim 1-2, wherein the sample is a microorganism, such as a virus, fungi or bacteria.

4 Method according to any one of claim 1-3, wherein the sample is suspected to comprise at least two types/species/targets of nucleic acid molecules chosen from the known set of types/species/targets.

5 Method according to claim 4, wherein the sample contains multiple infection/variants/types/species.

6 Method according to claim 5, wherein at least one primer is specific for a variant of a disease linked to the microorganism.

7 Method according to claim 6, wherein the microorganism is a human papillomavirus (HPV)

8 Method according to claim 7, wherein the known set of HPV-types are chosen from the group comprising the HPV-types; high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 68, 69 and low-risk: 6, 11, 34, 40, 42, 43, 44

9 Method according to any one of the preceding claims, wherein the sequence distance from the 3'-end of the general/consensus extendable primer could be avoided to be sequenced as in semi-conservative regions

10 Method according to any one of the preceding claims, for typing of amplicons comprising at least one semi-conservative region.

11 Method according to any one of claim 1-10 for typing of samples where a type/species is in minority or if the amplification fragment has low yield.

12 Method according to any one of claim 1-11, for typing/sequencing of samples containing unspecific amplification products, the primers of the set of primers not annealing to unspecific amplification products.

13 Kit for use in the method of typing of claim 1-12, comprising at least two oligonucleotide primers, whereby each primer is designed for being specific for one genotypes/type/species/group/target or type-specific region chosen from a known set of types of the type-specific/target region of a nucleic acid sample, thereby allowing a primer, which is specific for a genotype/type/species/target that is present in the sample, to hybridize in or close to the type-specific/target region or region of interest.

14 Kit according to claim 13, wherein the oligonucleotide primers of the kit are designed for being specific for any of the HPV-types chosen from the group comprising: high-risk: HPV-16, 18, 31, 33, 35, 39,45 , 51, 52, 58, 59, 66, 68, 69 and low-risk: HPV-6, 11, 34, 40, 42, 43, 44

15 Kit according to any one of the preceding claims for genotyping/typing/detection/identification/sequencing of any microorganisms, viruses or any other application where the multiple sequencing oligonucleotide primer pool approach is applicable by DNA sequencing technologies

16 Kit according to claim 1 for quantitative measurements of different genotypes/species/types amplified by PCR in the same sample or by mixing different amplicons

References

Berkenkamp, S., Kirpekar, F. and Hillenkamp, F.: Infrared MALDI mass spectrometry of large nucleic acids. Science 281 (1998) 260-262.

Fernandez-Contreras, M.E., Sarria, C., Nieto, S. and Lazo, P.A.: Amplification of human genomic sequences by human papillomaviruses universal consensus primers. J Virol Methods 87 (2000) 171-5.

Gharizadeh, B., Ghaderi, M., Donnelly, D., Amini, B., Wallin, K.L. and Nyren, P.: Multiple-primer DNA sequencing method. Electrophoresis 24 (2003) 1145-51.

Gharizadeh, B., Kalantari, M., Garcia, C.A., Johansson, B. and Nyren, P.: Typing of human papillomavirus by pyrosequencing. Lab Invest 81 (2001) 673-9.

Hagmar, B., Kalantari, M., Skyldberg, B., Moberger, B., Johansson, B., Walaas, L. and Warleby, B.: Human papillomavirus in cell samples from Stockholm Gynecologic Health Screening. Acta Cytol 39 (1995) 741-5.

Nordstrom, T., Nourizad, K., Ronaghi, M. and Nyren, P.: Method enabling pyrosequencing on double-stranded DNA. Anal Biochem 282 (2000a) 186-93.

Nordstrom, T., Ronaghi, M., Forsberg, L., de Faire, U., Morgenstern, R. and Nyren, P.: Direct analysis of single-nucleotide polymorphism on double-stranded DNA by pyrosequencing. Biotechnol Appl Biochem 31 (Pt 2) (2000b) 107-12.

Pourmand, N., Elahi, E., Davis, R.W. and Ronaghi, M.: Multiplex Pyrosequencing. Nucleic Acids Res 30 (2002) e31.

Ronaghi, M.: Improved performance of pyrosequencing using single-stranded DNA-binding protein. Anal Biochem 286 (2000) 282-8.

Ronaghi, M., Uhlen, M. and Nyren, P.: A sequencing method based on real-time pyrophosphate. Science 281 (1998) 363, 365.

Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74 (1977) 5463-5467.

Skyldberg, B., Kalantari, M., Karki, M., Johansson, B., Hagmar, B. and Walaas, L.: Detection of human papillomavirus infection in tissue blocks by in situ hybridization as compared with a polymerase chain reaction procedure. Hum Pathol 22 (1991) 578-82.